ORIGINAL ARTICLE

Investigation of cream and ointment on antimicrobial activity of *Mangifera indica* extract

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J. Adv. Pharm. Technol. Res.

ABSTRACT

Medicinal plants have curative properties due to the presence of various complex chemical substance of different composition, which are found as secondary plant metabolites in one or more parts of these plants. *Mangifera indica* Linn (MI L.) is a species of mango in the *Anacardiaceae* family. Phytoconstituents in the seed extracts may be responsible for the antimicrobial activity of the plant. The purpose of the study was to formulate and evaluate the antimicrobial herbal ointment and cream from extracts of the seeds of mango (MI L.) The formulated ointments containing oleaginous-based showed the best formulation compared to the emulsion water in oil type, the ointment and cream bases in different concentration 1%, 5% and 10%. The formulated ointment and cream of MI L. were subjected to evaluation of Uniformity of Weight, measurement of pH, viscosity, Spreadability, Acute skin irritation study, stability study and antimicrobial activity. Our study shows that MI has high potential as an antimicrobial agent when formulated as ointment and creams for topical use. Thus, the present study concludes that the formulated formulations of the MI are safe and efficient carriers, with potent antimicrobial activity.

Key words: Creams and antimicrobial activity, *Mangifera indica,* ointment

INTRODUCTION

Herbal medicine, also called botanical medicine or phytomedicine, refers to the use of any plant's seeds, berries, roots, leaves, bark, or flowers for medicinal purposes.[1] Long practiced outside of conventional medicine, herbalism is becoming more mainstream as up-to-date analysis and research show their value in the treatment and prevention of disease. Recently, the World Health Organization estimated that 80% of people worldwide rely on herbal medicines for some aspect of their primary healthcare.[2] Plant drugs are frequently considered to be less toxic and freer from side effects than the synthetic ones.[3] Along with other dosage forms, herbal drugs are also formulated in the form of ointment and creams. Medicated ointments contain a medicament dissolved, suspended or emulsified in the base. Ointments are used topically for several purposes, example, as protectants, antiseptics, emollients, antipruritic, keratolytics and astringents.[4] Ointment bases are almost always anhydrous and generally contain one or more medicaments in suspension or solution or dispersion. Ointment bases may be hydrocarbon (oleaginous), absorption, water removable and water soluble type.[5] The delivery of drugs through the skin has long been a promising concept because of the ease of access, large surface area, vast exposure to the circulatory and lymphatic networks and noninvasive nature of the treatment.[6].*Mangifera indica* (MI), also known as mango, has been an important herb in the Ayurvedic and indigenous medical systems for over 4000 years.[7] MI *Linn* (L.) belongs to the family *Anacardiaceae*; the tree is a native of tropical Asia and is indigenous to the Indian subcontinent, though it is now completely naturalized in many parts of the tropics and subtropics.[8] According to Ayurveda, varied medicinal properties are attributed to different parts of the mango tree. The presence of phytoconstituents in the leaf and seed extracts may be responsible for the antibacterial activity of the plant.[9] There are scanty reports on the activity of MI in the form of herbal ointment preparation to the best of the

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Access this article online

Quick Response Code:

Website: www.japtr.org

DOI: 10.4103/2231-4040.154530

authors' knowledge.[10] The objective of the study was to formulate and evaluate the antimicrobial herbal ointment and cream from extracts of the seeds of mango (MI L.).

MATERIALS AND METHODS

Plant collection and identification

Fresh seeds of MI L. were collected from the wild from around Khartoum vegetable, Sudan and were identified and authenticated by Dr. Hyder elshik, Research Center for Medicinal and aromatic plants and toxic, Sudan. The Sudanese MI L. was identified as mango. The collected seeds were kept at plastic bags at room temperature till use.

Extraction of mango seeds

Thousand gram of air dried and coarsely powdered of clean seeds of MI seed were extracted in Soxhlet apparatus to obtain methanolic and ethanolic extracts. The extracts were filtered, and the filtrates were vaporized to dryness, and weighed in order to determine the percentage yield of the extracts, following the formula:

% yield = (weight of extract/weight of ground plant material) ×100

The stock solutions of the crude ethanolic and ethanolic extracts were prepared by dilution the dried extracts with 50% methanol and ethanol, respectively, to obtain the desired final concentrations of: 5 mg/mL, 3.75 mg/mL, 3.125 mg/mL, 2.5 mg/mL, 1.875 mg/mL and 1.25 mg/mL. These concentrations were used to impregnate filter paper disks (5.5 mm diameters). Disk impregnated into 50% methanol, and 50% ethanol was used as control while standard antimicrobial discs: Amikacin, chlorophenicol, oxacillin, amoxicillin, nystatin and metronidazole (Difco) were used as positive control.

Preparation of extracts

The extraction was carried out using methanol and ethanol (separately). 400 g of dry seeds of MI fruit were extracted with 80% methanol using Sox-8 h till the color of the solvent returned colorless. Solvent was evaporated under reduced pressure using the Rotary evaporator apparatus (BUCHI Rotavapor R-200/20). Extract was finally allowed to dry at air at room temperature till complete dryness. Extraction using ethanol followed the above procedures.

Determination of phytochemical constituents

Preliminary phytochemical analysis was undertaken using standard qualitative methods.[11] The different crude extracts obtained were qualitatively tested for the presence of various phytochemical constituents using standard protocols.[12] The extractive values of mango were calculated as per standard methods.[12] The concentrated crude extracts obtained through were weighed and dissolved in the respective solvent used for the extraction (1 g/100 mL, w/v).

Formulation of creams and ointments

Mangifera indica fruit seed after the extraction process was formulated as creams and ointment by different concentration 1%, 5%, and 10%. Selection of oleaginous base for the formulation based on the preliminary studies showed the best formulation compared to the emulsion water in oil (w/o) type, the ointment and cream bases in different concentration 1%, 5% and 10% were selected as the final base for the preparation of ointment.

Evaluation

The above formulated ointment and cream of MI L. were subjected to evaluation for the following parameters as per the method described.[13]

Physical evaluation of the formulation

The formulations were inspected visually for their color, homogeneity, consistency, and phase separation.

Measurement of pH

The pH was measured using a pH meter, which was calibrated before each use with standard buffer solutions at pH 4, 7, 9. The electrode was inserted into the sample 10 min prior to taking the reading at room temperature.[14]

Uniformity of weight

A total of 10 bottles were filled randomly and weighed. Ointment and creams were removed from each bottle, and each empty bottle was washed with methanol. The empty bottles were dried, and their weight was taken. The difference between two weights was calculated as net weight of the ointment and cream of bottle. The average of net weight of ointment and creams of 10 bottles was noted.

Average bottle content = Total content of 10 bottles/No of bottles

Viscosity

The viscosity was determined by CAP-2000 Brookfield viscometer. Test sample was taken in a clean and dry 250 ml beaker, and the viscosity of the test sample was determined by standard operating procedure of Viscometer using spindle nos. 1–4. Each spindle was used for finding the viscosity of the sample at speeds of 0.3, 0.6, 1.5, 3, 6, 12, 30 and 60 r.p.m., respectively. Their rheological characteristics were also tested at 250 C using Brookfield viscometer.[15]

Spreadability

Spreadability is expressed in terms of time in seconds taken by two slides to slip off from the gel when placed in between the slides under the direction of a certain load. The excess amount of sample was placed between the two glass slides and a definite amount of weight was placed on these glass slides to compress the glass slides of uniform thickness.[16] A weight of 70 g was added and the time

required to separate the two slides was noted. Spreadability was calculated using the formula.

S = M.L/T

Where, M = wt. tied to the upper slide, L = length of glass slides,

T = time taken to separate the slides.

Acute skin irritation study

The primary skin irritation test was performed on male albino rats, weighing about 150–200 g. A set of six rats was used in the study for each formulation. The animals were maintained on standard animal feed and free access to water. The animals were kept under standard laboratory conditions. The dorsal hairs on the back of the rats were clipped off 1-day prior to the study. 50 mg of the different formulations was applied over a 1 cm area of intact skin on different animals. After the formulation was applied to the skin of rats, the animals were returned to the cages. After 48 h of exposure, the formulation was removed. The test site was wiped with tap water to remove any remaining residue. Undesirable skin changes, that is, change in color and changes in skin morphology were checked.

Stability study

All the developed formulations were subjected to accelerated stability testing for about 5 weeks. Room temperatures were maintained as per (ICH guidelines 1993). The parameter of formulation such as color, texture, Spreadability, pH, phase separation, skin irritation and viscosity were determined for all the formulations.

Antimicrobial evaluation using the disc diffusion method

The original extract of MI was subject to serial dilution as follows: 5 mg/ml, 3.75 mg/ml, 2.5 mg/ml, 1.75 mg/ml, and 1 mg/ml. Filter discs (5.5/mm) were made and impregnated into each of the above dilutions. The discs were dried at 37°C for 1 h. The dried discs were therefore having the following concentration: 5 mg/ml, 3.75 mg/ml, 2.5 mg/ml, 1.75 mg/ml, and 1 mg/ml. Muller Hinton's agar plates were used the *in vitro* antimicrobial testing as recommended by clinical and Laboratory Standards Institute.[17] Bacterial strains were sub cultured from frozen stocks or from plates freshly obtained from Asser Central Hospital. One of three loopful of 24 h old cultures from each test strains were used to prepare 0.5 MeFard standard suspensions.

Each bacteria strain (*Escherichia coli*, *Staphylococcus aureus*, *Candida albicans* and *Mycobacterium smegmatis*) were inoculated into Mueller-Hinton agar (MHA) plates to form a bacterial lawn. Then impregnated dried discs plus positive and negative control discs were placed on the inoculated MHA agar.

The inoculated plates were incubated at 37°C and examined after 24, 48 and 72 h for inhibition zones around the wells. Zones were measured in mm by a ruler for each disc exhibiting inhibition zones under and around discs.

RESULTS AND DISCUSSION

The MI fruit seed after the extraction process was formulated as creams and ointment with different concentration 1%, 5%, and 10%. The formulated ointments containing oleaginous base showed the best formulation. Due to the hydrocarbon properties of oleaginous bases they increase skin hydration by reducing the rate of water loss from the skin compared to the emulsion water in oil type, the ointment and cream bases in different concentration 1%, 5% and 10% were selected as the final base for the preparation of ointment because these bases were found to be compatible with the extracts at 10% concentration as well as possessing other optimum characteristics such as rate of release of medicament.

Batches of prepared creams and ointments formulations showed three different colors: Light brown, brown and dark brown depending on the different concentrations of active ingredient which was used in this study with a smooth and homogenous appearance, as given in Table 1. Uniformity of Weight for the average results of 10 individual readings were found to meet the release limit for the assay (British Pharmacopeia., 2012), Shown in Table 2. The pH of all of the MI extracts formulations was found to be in the range of 5.8–6.3 for the creams and in the range of 5.6–6.9 for the ointments, which lies within the normal pH range of the skin. The results obtained showed that the pH of the formulations was decreased when duration of storage increased as shown in Table 3.

Prepared formulations possessed optimum viscosity. Since, the type and quantity of the cream and ointment in each

Table 1: Homogeneity of cream and ointment formulations

Formulation (%)	Color	Homogeneity	Appearance
FC1 (1)	Light brown	Homogenous	Smooth, opaque, glossy and greasy on application
FC2 (5)	Brown	Homogenous	Smooth, opaque, glossy and greasy on application
FC3 (10)	Dark brown	Homogenous	Smooth, opaque, glossy and greasy on application
FO1 (1)	Light brown	Homogenous	Smooth, opaque, glossy and greasy on application
FO2 (5)	Brown	Homogenous	Smooth, opaque, glossy and nongreasy on application
FO3 (10)	Dark brown	Homogenous	Smooth, opaque, glossy and nongreasy on application

FC: Formula of cream, FO: Formula of ointment

formulation was the same, inclusion of different content seems to have brought about some difference in the viscosity of the cream and ointment. While FO3 was the most viscous formulation, FC1 had the least viscosity [Table 4]. The values of the spreadability indicated that the MI extracts formulation were easily spreadable by a small amount of shear. Formulation FC3 gave the highest value for spreadability [Table 5]. The skin irritation study was performed on male albino rats. This test is significant as it gives an idea about the emollient properties of the prepared formulations against the skin irritation caused by MI seed extract. The results of skin irritation were assessed by visual inspection. They are given in Table 6. The parameter of formulation such as color, texture, Spreadability, pH, phase separation, skin irritation and viscosity were satisfactory and acceptable for all formulation [Table 7]. Results obtained from the antimicrobial study showed that all developed formulations have an inhibitory effect on the *S. aureus*. The *in vitro* antimicrobial activities were calculated in term of the zone of inhibition diameter (cm) the result recorded in [Table 8]. FO3 showed the higher zone of inhibition against *S. aureus, C. albicans, E. coli, M. smegmatis*. Formulation FC1, FC2, FC3 of cream have lesser zone of inhibition compared with FO1, FO2 and FO3 of ointment.

Table 2: Uniformity of weight

Bottle number	Bottle content (mg)	91%-109%	√ or X
1	210	97	√
2	211	96.5	√
3	196	103.9	√
4	206	98.8	√
5	204	99.8	√
6	201	101.3	√
7	212	96	√
8	223	91.3	√
9	213	95.6	√
10	197	103.4	√
Average	203.7		

Average bottle content=Total content of 10 bottle/number of bottles

Table 3: pH of creams on storage

Formula	Temperature (°C)	Time (in days)				
		Initial	14	21	28	35
pH measurement						
FC1	25	6.37	6.26	6.18	5.98	5.88
FC2	25	6.34	6.24	6.9	6.05	5.84
FC3	25	6.28	6.20	6.2	5.97	5.92
FO1	25	6.98	6.60	6.56	6.37	5.91
FO2	25	6.91	6.65	6.55	6.20	5.78
FO3	25	6.89	6.45	6.51	6.14	5.68

FC: Formula of cream, FO: Formula of ointment

Table 4: Viscosity of creams and ointments formulated with *Mangifera indica*

Formulation (%)	Temperature (°C)	Time (in days) Initial	90 days (cp)
FC1 (1)	25	81	81.2
FC2 (5)	25	81.1	82
FC3 (10)	25	81.2	83.1
FO1 (1)	25	83.3	83
FO2 (5)	25	83.2	83.5
FO3 (10)	25	83.7	83.9

FC: Formula of cream, FO: Formula of ointment

CONCLUSION

This study shows that MI has high potential as antimicrobial agent when formulated as ointment and creams for topical use and could, therefore, explain the successes claimed in the folk use of the plant in the treatment of common skin conditions. Among the Prepared Formulation Batches, FO3 showed the higher zone of inhibition against *S. aureus, C. albicans, E. coli, M. smegmatis*. Formulation FC1, FC2, FC3 of

Table 5: Observations of the spreadability of *Mangifera indica* extracts formulation

Formulation (%)	T	Spreadability (g.cm/s)
FC1 (1)	18.5	202.7
FC2 (5)	18.2	206.04
FC3 (10)	17.7	211.86
FO1 (1)	21.6	173.6
FO2 (5)	21.2	176.88
FO3 (10)	20.7	181.15

FC: Formula of cream, FO: Formula of ointment=Weight applied over the slide=Length of the slide, T=Time required to move the slides

Table 6: Observation from skin irritation study

Formulation (%)	Observation
FC1 (1)	No sign of redness or dryness observed
FC2 (5)	No sign of redness or dryness observed
FC3 (10)	No sign of redness or dryness observed
FO1 (1)	No sign of redness or dryness observed
FO2 (5)	No sign of redness or dryness observed
FO3 (10)	No sign of redness or dryness observed

FC: Formula of cream, FO: Formula of ointment

Table 7: Accelerated stability studies of *Mangifera indica* extracts formulation

Characteristics of the formulation	(F) type	Time (in days)				
		Initial	14	21	28	35
pH measurement	FC	6.37	6.26	6.18	5.98	5.88
	FO	6.98	6.60	6.56	6.37	5.91
Viscosity (cp)	FC	81.1	83.6	83.4	83.3	82.3
	FO	83	83.3	82	83.6	82.8
Spreadability (s), $n=5$	FC	22	20	20	19	19
	FO	21	20	19	20	20
Zone of inhibition (mm)	FC	6	6.7	5.2	5	4
	FO	8	9.2	7.1	6.2	5.3

FC: Formula of cream, FO: Formula of ointment

Table 8: Anti-microbial activity of pharmaceutical formulation for microorganism with agar well diffusion method

Microorganism	Cream formulation (mm) (%)			Ointment formulation (mm) (%)			Extract of *Mangifera Indica* (mm)		
	FC1 (1)	FC2 (5)	FC3 (10)	FO1 (1)	FO2 (5)	FO3 (10)	P.E.M	P.E.E	C
Escherichia coli	5	7	10.2	5.3	7.6	11.8	6	10	Growth
Staphylococcus aureus	4.1	8	8.9	4.6	9.1	12.2	10	10	Growth
Candida albicans	4.2	6.1	8.3	5.1	8.3	13.4	12	11	Growth
Mycobacterium smegmatis	4.6	7.6	9.1	5.2	8.2	11.2	8	18	Growth

FC: Formula of cream, FO: Formula of ointment, P.E.M: Pure extract of *Mangifera indica* 5% with methanol, P.E.E: Pure extract of *Mangifera indica* 5% with ethanol, C: Control

cream have lesser zone of inhibition compared with FO1, FO2, and FO3 of ointment. The formulated formulations showed acceptable physical properties, and hence, were compatible with the skin. In addition, the formulated formulations passed the short-term stability, indicating the physical and chemical stability of the product. Hence, the formulated formulations of the MI were safe and efficient carriers, with potent antimicrobial activity.

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How to cite this article: Awad El-Gied AA, Abdelkareem AM, Hamedelniel EI. Investigation of cream and ointment on antimicrobial activity of *Mangifera indica* extract. J Adv Pharm Technol Res 2015;6:53-7.

Source of Support: Nil, **Conflict of Interest:** Nil.